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Abstracts From the 69th Scientific Sessions
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Named and Invited Lectures

Lewis A. Conner Memorial Lecture • Sol Sherry Lecture in Thrombosis • Thrombosis Distinguished Lecture • Paul Dudley White International Lecture • George Lyman Duff Memorial Lecture • Lewis K. Dahl Memorial Lecture • William W.L. Glenn Lecture • Ancel Keys Lecture • Dickinson W. Richards Memorial Lecture • Laennec Society Invited Lecture • Charles T. Dotter Memorial Lecture • Alexander S. Nadas Lecture • George E. Brown Memorial Lecture

Young Investigator Award/Prize Abstracts

The Council on Cardiovascular Nursing New Investigator Awards • Melvin L. Marcus Young Investigator Awards in Cardiovascular Integrated Physiology • Samuel A. Levine Young Clinical Investigator Awards • Louis N. and Arnold M. Katz Basic Science Research Prizes for Young Investigators • The Cournand and Comroe Young Investigator Prizes in Cardiopulmonary and Critical Care • Irvine H. Page Arteriosclerosis Research Awards for Young Investigators • Young Investigator Prizes in Thrombosis • Elizabeth Barrett-Connor Research

was not associated with increases in TF mRNA. Furthermore, the increase in TF activity by hydrogen peroxide, unlike that induced by ox-LDL or FBS, was not inhibited by cycloheximide or brefeldin A, indicating that neither protein synthesis nor trafficking through the golgi apparatus was necessary. Consistent with these observations, western blot analysis demonstrated an increase in TF protein by ox-LDL and FBS, but no increase after hydrogen peroxide. To determine if the increase in TF activity induced by hydrogen peroxide was due to activation of latent protein on the surface, or translocation of intracellular protein to the surface, we performed immunoprecipitation of surface TF by incubating cell monolayers with anti-TF antibody at 4°C after exposure to hydrogen peroxide. Western blot analysis showed no increase in surface TF protein. These data demonstrate that oxidant stress increases TF activity at the SMC surface and may contribute to thrombosis after vascular reperfusion or arterial lesion development.

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INTERACTION OF DIABETIC ERYTHROCYTES BEARING ADVANCED GLYCATION ENDPRODUCTS WITH THE ENDOTHELIAL RECEPTOR AGE INDUCES GENERATION OF REACTIVE OXYGEN INTERMEDIATES AND CELLULAR DYSFUNTION.

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Diabetic red blood cells (RBCs) undergo nonenzymatic glycation leading to formation of cell surface Advanced Glycation Endproducts (AGEs). Such diabetic RBCs display increased adherence to endothelial cells (ECs) via the immunoglobulin superfamily receptor RAGE (Receptor for AGE), resulting in increased vascular permeability. Although, diabetic RBCs do not produce reactive oxygen intermediates (ROIs), following binding to ECs labelled with the fluorescent probe DCF-DA or DHR, generation of hydrogen peroxyde is observed in the endothelial monolayer. Production of ROIs was blocked by diphenyl iodonium (DPI), whereas it was unaffected by inhibitors of nitric oxyde synthase, suggesting that activation of NADPH oxydase had occured as a result of diabetic RBCs binding to ECs. Addition of anti-RAGE IgG or excess of soluble extra cellular domain of the receptor prevented diabetic RBC- EC interaction, and blocked production of ROIs. Consequences of diabetic RBC-induced EC oxidant stress included increased expression of Interleukin 6 and Vascular Cell Adherence Molecule-1, both of which were prevented by antioxidants or blockade of RAGE. These results indicate that ligation of RAGE by diabetic RBCs activates intra cellular pathways leading to the formation of ROIs, indicating a means through which AGEs on the surface of circulating blood cells perturb the endothelium in diabetes.

Arteriosclerosis/Basic Science/Cardiopulmonary and Critical Care/Circulation/Thrombosis:

Oxidant Stress in Cardiovascular Pathophysiology
Wednesday Afternoon
Exhibit Hall
Abstracts 4140–4152

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Alterations in β_1-Subunit of the Enzyme as a Mechanism of Depression in the Cardiac Sarcolemmal Na^+-K^+ ATPase Due to Hypochlorous Acid (HOCl)

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Oxidative stress under in vivo pathophysiological conditions such as ischemia- reperfusion is known to be associated with the formation of HOCl and depression of the cardiac sarcolemmal (SL) Na^+-K^+ ATPase activity. Although HOCl has been shown to produce a dramatic decrease in the SL Na^+-K^+ ATPase activity, the mechanisms for this change are poorly understood. In this study, SL membranes were isolated and purified from the porcine heart and the effects of different concentrations (0.02 to 0.4 mM) of HOCl were examined. The kinetic characterization of the SL Na^+-K^+ ATPase in the presence of different concentrations of MgATP revealed a decrease in V_{max} value (20.0 ± 1.75 vs. 6.7 ± 1.70 μmol Pi/mg/hr) without any change in K_m (0.33 ± 0.04 0 vs 0.40 ± 0.08 mM MgATP) due to treatment of membranes with 0.1 mM HOCl. When the Na^+-K^+ ATPase activity in HOCl-treated SL membranes was determined in the presence of different concentrations of Na^+, a depression in the V_{max} value similar to that seen with MgATP was observed, but the affinity of HOCl-treated Na^+-K^+ ATPase for Na^+ was increased because the K_a value was decreased from 14.3 ± 2.7 to 1.4 ± 0.1 mM. The sulfhydryl (SH) group content of the SL membrane was decreased (28.7 ± 4.4 vs 64.8 ± 6.3 nmol/mg) whereas the malondialdehyde (MDA) content was increased (67 ± 3.4 vs 51 ± 3.9 nmol/mg) upon treatment with 0.1 mM HOCl. Scatchard plot analysis of the ^{3}H-ouabain binding data indicated no significant ($P > 0.05$) change in K_d (8.9 to 10.3 nM) or B_{max} (49 to 50 pmol/mg) values upon treating SL membranes with HOCl. The Western blot analysis of the Na^+-K^+ ATPase subunits in the HOCl-treated SL membranes showed a marked decrease (68 ± 3.2% of control) in β_1-subunit without any change in α_1- or α_2-subunits. The HOCl-induced alterations in the structure-function parameter of SL Na^+-K^+ ATPase as well as SH-group and MDA contents in the SL membrane were prevented by the presence of both L-methionine and dithiothreitol in the incubation medium. These data suggest that the HOCl-induced depression in the SL Na^+-K^+ ATPase activity may be due to changes in the β_1-subunit of the enzyme.

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Rac1 regulates [illegible]ne-stimulated, redox-dependent pathway necessary for NF-κB activation and VCAM-1 expression

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The signal transduction pathway leading to the activation of the transcription factor NF-κB remains incompletely characterized. We demonstrate that in HeLa cells, transient expression of a constitutively active mutant of the small GTP-binding protein rac1 (V12rac1) leads to a significant increase in NF-κ B transcriptional activity. In addition, expression of a dominant negative rac1 mutant (N17rac1) inhibits basal and IL-1β stimulated NF-κB activity. Gel shift analysis using nuclear extract prepared from HeLa cells infected with a recombinant adenovirus encoding N17rac1 (Ad.N17rac1) showed reduced levels of cytokine-stimulated DNA binding to a consensus NF-κB binding site. The ability of V12rac1 to stimulate NF-κB activity appears independent of the ability of rac proteins to stimulate the family of c-jun amino terminal kinases (JNKs). In an effort to explore how rac proteins regulate NF-κB activity, we demonstrate that expression of V12rac1 or stimulation with cytokine results in a significant increase in intracellular reactive oxygen species (ROS). The rise in ROS which occurs following V12rac1 expression can be blocked by two chemically unrelated antioxidants. Treatment of HeLa cells with antioxidants also inhibits the ability of V12rac1 to stimulate NF-κ B activity. In addition we provide evidence that rac proteins regulate NF-κB activity in other cell types. Previous studies have demonstrated that endothelial expression of VCAM-1 results from the redox dependent activation of NF-κB . We demonstrate that adenoviral-mediated gene transfer of N17rac1 into endothelial cells results in inhibition of VCAM-1 expression. These results suggest that rac1 regulates intracellular ROS production and that rac proteins function in a redox-dependent signal transduction pathway leading to NF-κB activation.

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REGULATION OF EXTRACELLULAR MATRIX COMPONENTS AND VASCULAR TONE BY HOMOCYSTINE AND OXIDATIVE MIXED-DISULFIDES IN HUMAN ATHEROSCLEROTIC LESIONS,

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Prevalence of hyperhomocyst(e)inemia in the plasma of patients with peripheral arterial occlusion has been demonstrated. Oxidative mixed disulfide homocystine induces endothelial cell dysfunction and promotes growth of vascular smooth muscle cells. We have demonstrated that the reduction-oxidation (redox)-state regulates extracellular matrix (ECM) components in human cardiovascular cells (Tyagi et al, 1996, *J Cell Biochem* , 61 , 139-151). The role of tissue, protein bound and unbound, oxidative mixed disulfides in the development of fibrous plaque in atherosclerotic lesion is not known. Atherosclerotic coronary arteries were isolated from ischemic explanted hearts. Internal mammary arteries were used as normal vascular tissue control. Tissue extract from atherosclerotic lesions and normal arteries devoid of adventitia were prepared. Interaction of homocystine with Ellman's reagent (5, 5ν-dithio-bis-2-nitro benzoic acid) catalyzed by reducing agent generated changes in optical density (OD) at 405 nm in a dose dependent fashion. Changes in OD with increasing amounts (0-25 μg) of homocystine demonstrated linearity in standard curves. Tissue extracts from atherosclerotic lesions (n = 20) and normal vessels (n = 10) were prepared. Protein-bound oxidized disulfides were precipitated by trichloroacetic acid (TCA) and supernatant collected for free-oxidative disulfides. Protein-bound and free disulfide levels were 1.0 ± 0.3 and 1.5 ± 0.2 μg/mg in atherosclerotic tissue; and 0.1 ± 0.01 and 0.12 ± 0.02 μg/mg in normal tissue, respectively. Results suggest 10-fold increase ($p < 0.01$) in protein-bound and 15-fold increase ($p < 0.01$) in free oxidative disulfide in atherosclerotic tissue as compared to normal tissue. Isometric measurements in normal coronary rings demonstrate vasoconstrictor effects of homocystine. To determine the role of homocystine in ECM expression, normal medial human vascular smooth muscle cells (HVSMC) were isolated from internal mammary artery. Northern-blot analysis using type I collagen cDNA probe and densitometric analysis on mRNA isolated from HVSMC with and without the treatment of 10 μM of homocystine was performed. Collagen type I mRNA was increased 5-fold ($p < 0.005$) in treated versus untreated cells. Results suggest increased tissue level of oxidative disulfides and a role of disulfide homocystine in the development of fibrous atherosclerotic plaque and altered vascular function.

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Gender But Not Antioxidants Affects the Reduced Susceptibility to Peroxidation of Plasma Lipids Following a Single Bout of Ultraendurance Exercise.

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Exercise reduces the risk of coronary artery disease (CAD) in men and women and paradoxically, may promote free-radical formation, lipid oxidation and vascular tissue injury. Using the Hawaii Ironman Triathlon as a model for exercise-induced oxidative stress, 39 athletes (26 males, 13 females, mean age 38±10 yrs) were studied 24 hours prior to and 15 min after a 2.4 mi swim, 112 mi bike, 26.2 mi run consecutively. Lipid and antioxidant measurements were performed in a CDC standardized laboratory; susceptibility of plasma lipids to peroxidation (Perox) was measured using the Fe^{+2}-H_2O_2 method. Mean±SD were compared using a two-tailed t-test. Lipid changes were favorable and were no different for men vs. women. Surprisingly, a marked reduction in Perox was observed after exercise, which was not associated with antioxidant use or levels but which was weakly correlated with reduction in serum iron ($p < 0.05$). Moreover, a striking difference was seen in the changes Perox following exercise in men vs. women. Thus ultraendurance exercise 1) favorably alters lipid factors causally associated with CAD, and 2) decreases Perox in men but not in women potentially indicating an effect on Perox of exercise-induced changes in endogenous hormones.